Date: November 14, 2001

To: BLA STN 125029 file

From: Gibbes Johnson, Ph.D, Frederick Mills, Ph.D.

Through: Amy Rosenberg, M.D., Barry Cherney, Ph.D.

Re: Review of Eli Lilly and Company's Response to the Agency's CMC Discipline Review Letter; Amendment 24, 33 and 38 (sponsor submissions dated 10-9-01, 10-24-01 and 11-8-01) Submitted to BLA STN 125029; Xigris, Drotrecogin alfa; Activated Protein C (APC)

Question 1a

The defined lifespan for each commercial scale chromatography column and filter used in the purification of drug substance and information attesting to how the lifespan was established.

Question 1b

Please provide information which confirms the ability to clean the filter or columns and associated equipment (i.e. injectors, etc.) over the defined lifespan.

Lilly Response:

Capture Column Lifetime and Resin Reuse

The capture chromatography column will not be subjected to more than --- elution cycles. Resin from a column subjected to --- cycles showed comparable performance at the laboratory scale to chromatography on new resin. ---- cycles exceeding --- runs were demonstrated at the pilot scale in the production of clinical trial material. Another cycle of --- runs was demonstrated at the commercial scale in a development facility. Each of these systems generated mainstreams that met all of the criteria for forward processing, a reproducible chromatographic profile, and no significant changes in pressure-flow relationships. ---------- analysis of the mainstream fractions shows there is no significant alteration of the ---------- profile with column use up to --- elution cycles at

the pilot scale. Resin subjected to --- cycles at the commercial scale was used in viral clearance studies and gave rise to the same levels of viral clearance as new resin. Suitability of the cleaning regimen is demonstrated by the ----------, ----------. Viral inactivation by the regeneration solutions is discussed in the viral safety assessment. Resin subjected to --- cycles and loaded with a ---------- containing no product confirmed the absence of product in the mainstream elution fraction.

Suitability of resin reuse has been confirmed in the manufacturing facility. Capture columns were subjected to --- runs during the execution of the consistency protocol at ---------- and then subjected to an additional ----- runs (--- total) prior to repacking. This full-scale data confirmed consistency of performance throughout the column lifetime. At the end of the consistency runs and after --- cycles, commercial columns were loaded with a ---------- and the simulated mainstream fractions were evaluated. The absence of ---------- was confirmed in the simulated mainstream by both ---------- measurements, ---------- analysis, product specific ---------- assays, and ----------. Simulated mainstreams were also confirmed to contain no ---------- assay. The consistent performance of the column throughout --- cycles in laboratory, pilot scale, and commercial settings, as well as data from the simulated mainstreams support the effectiveness of the cleaning methods used for sanitization/ regeneration following product runs on the ---------- column and justify the reuse of this column for --- elution cycles.

------- Column Lifetime and Resin Reuse

The ---------- and ---------- Fast Flow columns are used in sequence as a tandem chromatography operation. The ---------- column is used ------ and discarded. ---------- ---------- resin will not be subjected to more than --- elution cycles. Laboratory scale studies have confirmed that ---------- ---------- resin subjected to --- elution cycles generates a mainstream that meets all of the criteria for forward processing and a reproducible chromatographic profile. ---------- ---------- have been shown to be acceptable through --- elution cycles. Blank

runs with buffer (----------) over used resin confirm the absence of product in the mainstream elution fraction, demonstrating suitability of cleaning. Viral clearance has also been demonstrated to be unaffected by using resin subjected to --- elution cycles. At this time, resin performance has been confirmed in the commercial setting up to ------ runs by the criteria of conformance to all of the critical process parameters and criteria for forward processes described in the initial BLA.

A protocol to confirm resin reuse in the manufacturing facility up to --- elution cycles using ---------- is in progress. It is anticipated that the protocol will be completed by the second quarter of 2002. At the end of the consistency runs and after --- cycles, commercial columns were loaded with a ---------- and subjected to commercial wash/elution cycles. A simulated mainstream fraction was collected ---------- and analyzed relative to buffer controls to provide an interim analysis of column performance. The absence of ---------- was confirmed in simulated mainstreams by ---------- measurements, ---------- assay, and ----------. In summary, no accumulation of ---------- was observed in simulated mainstreams derived from used resin. Simulated mainstreams were also confirmed to contain no ---------- assay. The data currently available confirm, at the commercial scale, the suitability of resin reuse for up to --- cycles.

---------- Membrane Lifetime and Justification for Membrane Reuse

The ---------- Membrane will not be used for more than --- cycles. Suitability for use is determined prospectively based upon ---------- ----------. Before and after each use in the commercial setting, a ------- -------- test is performed with the system still assembled and all membranes in place as described in the marketing application. If the ----------

observed is less than ---------- of the value obtained for new membranes or less than ----- of the pre-run test result, the membranes are ----------.

As noted in the initial BLA, the drop in ---- relative to new membranes is not a result of ---------- as it is also observed in runs made in the absence of product. Vendor product information suggests that this is most likely due to ----------.

Laboratory studies with representative process streams and membranes have confirmed the suitability for reuse through ----- cycles of product ---------- by demonstrating no change in ---------- at either beginning of ----------, the end of ----------, or the end of ----------

Additional confirmation of appropriate cleaning is demonstrated by analysis of laboratory membrane systems subjected to ---- cycles of product ---------- showing less than ---------- in a post-use rinse. No differences were observed between membranes subjected in the laboratory to ---------- cycles of product ---------- following destructive testing and ---------- analysis.

------ sets of ----- membranes were subjected to a simulated run using both ----------. Samples assayed represent rinses of both the membranes and the product contact surfaces of the equipment skid.

None of the rinses from each of the ------ membrane sets showed any indication of ----------. Moreover, DNA threshold analyses of the rinses indicated less than the limit of quantitation for the assay in all cases.

These data confirm that membranes meeting the operational qualifiers defined for the ----- are suitable for reuse and do not accumulate ----------

---------- Filtration (Viral Clearance) Membrane Lifetime

The membrane cassettes used for the ---------- step are ----------.

Reviewer's Conclusion:

This response is acceptable.

Question 1c

In instances where a lifespan has yet to be established, how will the commercial scale lifespan be defined and how will the ability to clean the column or filter be evaluated over the defined lifespan? What will constitute a failure in the performance of the chromatography columns and filters in these studies? What will constitute a failure in the ability to clean the columns and filters in these studies?

Question 1d

In the case of a failure, how will the disposition of the lot(s) produced since the last passing evaluation be determined?

Lilly Response

The lifespan for the capture column, the ---------- filter, and the ---------- has been established at ----------., the commercial facility. The ---------- column from the ---------- chromatography step is used ----------.

Evaluation of ----- Column Lifetime in the Commercial Operations

At this time, resin performance has been confirmed in the commercial setting for the ---------- column for up to ---------- runs by the criteria of conformance to all of the critical process parameters and criteria for forward processes described in the initial BLA.

Analysis to determine residual protein, ---------- levels will be performed to --- elution cycles in the commercial setting. These data will confirm the suitability of column reuse up to limits defined in smaller scale studies. If, in the commercial setting, any of the critical process parameters noted in the BLA, Section I.D.1.b., In-Process Controls for Purification, were to be exceeded, the lot would be in deviation and disposition would await closing of the deviation investigation and report.

In the event that results from a blank run after --- cycles were to generate data which were significantly different from those seen to date, the lots in question would also be in deviation per ---------- SOP OP0028, “Deviation/Investigation Report.”
Eli Lilly and Company commits to provide the results of the resin lifespan study of the ---------- column to the FDA as required per 21 CFR 601.12(d),
“Changes to be described in an annual report (minor).” Should any deviation occur during the resin lifetime studies, the drug substance lots in question will be in deviation per ---------- SOP OP0028, “Deviation/Investigation Report.” In the event of such an occurrence, the results of the investigation would be submitted to the FDA per 21 CFR 601.12(c), “Changes requiring supplement submission at least 30 days prior to distribution of the product made using the change” prior to release of the drug substance lots in deviation.

Reviewer's Conclusion:

This response is acceptable.

Question 1e

Please provide any plans for extending the established lifespans of columns or filters.

Lilly Response

There are currently no plans to extend the established lifespans of the columns or filters used in the manufacture of recombinant human Activated Protein C drug substance. In the event that Eli Lilly and Company should plan to extend the column lifespans, studies will be conducted and the data will be submitted to CBER as a post-approval supplement to the BLA as required in 21 CFR 601.12(b)(3).

Reviewer's Conclusion:

This response is acceptable.

Question 2

Please provide information which confirms that the assays used for release testing of drug substance provide an assurance that all disulfide bonds in rhAPC have been correctly formed.

Lilly Response

Assurance that the disulfide bonds in rhAPC have been correctly formed is provided through extensive characterization of the rhAPC reference standard as well as lot release testing. The disulfide linkages present in the rhAPC reference standard Lot ---------- have been thoroughly characterized. The disulfide bonds of human protein C are formed within the ---------- and the protein is secreted ----------
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----------). Based on these factors, one would not expect the disulfide linkages to be impacted by process scale. To confirm this expectation both pilot-scale and full-scale lots of rhAPC have been assayed using ---------
----------. These data confirm that the expected disulfide linkages are present.

Additional assurance that the expected disulfide linkages are present is provided by ----------, ---------- lot release assays. -------- activity is dependent on the structures of both the ----------
----

---------- analysis will also detect significant

changes in ---------- resulting from disulfide bond alterations. In addition, ---------- will detect the ----------.

Reviewer's Conclusion:

This response is acceptable.

Question 3

Please confirm that all drug product lots intended to be released for commercial distribution were produced by the identical validated drug substance and drug product manufacturing process.

Lilly Response

All XIGRIS drug product lots intended to be released for commercial distribution were produced by the validated manufacturing process. All drug product lots intended for commercial ditribution were manufactured from recombinant human Activated Protein C drug substance lots that were produced by the validated manufacturing process.

Reviewer's Conclusion:

This response is acceptable.

Question 4

The BLA contained drug substance stability data for up to --- months at --- °C and --- months at --- °C. Based on these data, an expiration dating period of ---months at --- °C can be granted. Please provide a stability protocol for FDA review. Upon review and approval of this protocol, data supporting extension of the dating period can be submitted in an annual report.

Lilly Response

Storage of recombinant human Activated Protein C drug substance at ----------. is in a --------- with a setpoint of -----°C with a tolerance of approximately +/- 5°C. While the storage temperature for the drug substance is described in the initial BLA as "Less than or equal to ----°C," (Section I.G., Container Closure System), this represents a worst case scenario. In addition, ---------- month stability data at accelerated storage conditions -----°C) provides assurance that the drug substance remains stable during possible brief excursions above the ---------- setpoint of ----°C. Therefore, Eli Lilly and Company believes that the ---months long-term stability data (----°C setpoint with a tolerance of approximately ----------°C) for the primary drug substance lots submitted September 7, 2001, Serial No. --------, supports an expiration dating period for the drug substance of --- months. When --- month stability data is completed according to the stability protocol provided in Section I.H.1., Drug Substance Stability Protocol, page 799, Eli Lilly and Company will extend the expiry dating to --- months and submit the data in an annual report as required by 21 CFR 601.12(d)(2)(iii). In addition, at least one lot of drug substance will be placed on stability according to the Stability Protocol for Future Lots provided in Section I.H.1.a., Drug Substance Data, page 840.

Moreover, as noted above in the review of Drug Substance stability, the stability of rhAPC Drug Substance was investigated in a ----L pilot scale storage vessel which is representative of the commercial ----L storage vessel. The contents of the pilot vessel were thawed after --- and --- months of storage in a ------- maintained at ----°C. The data demonstrated that rhAPC Drug Substance is stable for at least --- months when stored at ----°C

Reviewer's Conclusion:

There is adequate justification for an --- month Drug Substance lifetime, and also an adequate proposal for extending the lifetime to --- months when data becomes available. This response is acceptable.

Question 5

The drug product manufacturing section of the BLA (page 90) contains a description of the ----------. Please submit to the BLA a validation study which supports this ---------- step and includes an analysis of drug product stability following such ----------.

Lilly Response

During the manufacturing of a 20 mg/vial full-scale development batch of rhAPC Drug Product (Batch --------) at ----------, the rhAPC Drug Product Solution was ----------. The purpose of this intentional ---------- was to demonstrate that ----------, in the event of ---------- during commercial manufacturing, does not affect the chemical properties of the rhAPC Drug Product Solution. ---- Full-scale rhAPC Drug Substance Lots (---------- Lots ----------) were used to manufacture rhAPC Drug Product Batch ----------.

These analyses indicate no product impact of refiltration.

In order to demonstrate that refiltration has no significant effect on stability, Lot 0H6007 was analyzed after 12-months of storage at ambient-room-temperature conditions. Room-temperature storage was taken to represent a "worst-case" situation. Lot 0H6007 was stored at controlled-refrigerated conditions of 2 - 8°C for the first 13 weeks and was then then transferred to an ambient-room-temperature storage area. The temperature of the ambient-room-temperature storage area is controlled at a setpoint of 18.3°C. The upper-alarm setpoint is 25°C and the lower-alarm setpoint is 15°C. The temperature typically ranges from 16.1°C to 23.9°C. Results of physicochemical analyses conducted at the initial time and at 12 months are shown in Table 2.

The results in Table 2 indicate the physical and chemical properties of rhAPC Drug Product Lot 0H6007 at the 12-month timepoint are not significantly different than those determined initially. The difference in potency values between the initial and 12-month results is not significant, because it is within one sigma (24 Units/mg) of assay variability. There was a slight increase in the moisture content of the vials. A slight increase is typically observed for rhAPC Drug Product stored at controlled-roomtemperature
conditions (25°C, 60% RH). Because the results demonstrate that refiltration
has no significantly effect the physical and chemical properties of the lyophilized rhAPC Drug Product, the rhAPC Drug Product Solution can be successfully refiltered during manufacturing of rhAPC Drug Product as a contingency to a sterilization-filter failure.

Eli Lilly and Company commits to placing ---- additional drug product lots requiring ---------- on stability studies as they occur. The stability data from these ---- lots will be submitted to the FDA as required per 21 CFR 601.12(d), "Changes to be described in an annual report (minor)." If acceptable stability data results are obtained, the ---------- process will be considered a validated process for those lots requiring ---------- in the event of a ----------.

Reviewer's Conclusion:

This response is acceptable.

Question 6

Please note that -- month drug product stability data on the 10 mg clinical formulation is not adequate to support --- month expiration dating for the commercial 5 mg and 20 mg formulations. Additional real time stability data for the 5 mg and 20 mg formuations submitted in your September 7, 2001 amendment is sufficient to support an --- month expiration date. Please submit a revised drug product stability protocol that provides for placing a least one lot of both the 5 mg and 20 mg presentations on stability each year. Upon review and approval of this protocol, data supporting extension of this dating period can be submitted in the annual report.

Lilly Response

When --- month stability data is collected from the primary stability study, from the protocol provided in Section II.H.1., Drug Product Stability Protocol, page 233, the dating for the 5 and 20-mg drug product presentations will be extended to a shelf-life of --- months. These data, supporting the dating extension, will be submitted in the annual report as required in 21 CFR 601.12(d)(2)(iii).

In addition, at least one drug product lot of both the 5 and 20-mg presentations will be placed on stability according to the Stability Protocol for Representative Lots provided in

Section II.H.2., Future Stability Protocol, page 342.

Reviewer's Conclusion:

This response is acceptable.

Question 7

Please specify the manufacturers of the ---------- and --------media used in cell banking, and supply Certificates of Analysis for these media.

Lilly Response

The raw materials ----- and -----, used in Cell Culture and Harvesting, Step Nos. 3 (Inoculum Bioreactor) and 4 (Production Bioreactor) are supplied by both ----- ----------. The Certificates of Analysis for the ---------- and ----------) from both suppliers are provided on pages 18-22 of Amendment 24.

Question 8a

Please adapt the ---------- identity test performed under ---------- for use as a purity assay. Please implement this assay for use in ---------- and drug product release testing and in ----------. This analysis should include an evaluation of the ---------- (----------

Lilly Response

The ---------- method will be revised and validated for use as a purity method. The new revision will be used for ----------

and drug product. The new test method will be implemented by September 1, 2002. In addition, the ---------- and drug product specifications will be revised to include the ---------- test as a purity method. This information will be submitted to the BLA as a "Supplement-Changes Being Effected" as required by 21 CFR601.12(c).

Reviewer's Conclusion:

This response is acceptable.

Question 8b

Please perform analysis of drotrecogin alfa (activated) ----------, in the drug substance and drug product stability studies to support the expiration dating. Please implement this analysis for use as a drug product release test.

Lilly Response

Data demonstrating ---------- stability has been obtained for both drug substance stored in the ---------- at ----°C for --- months as well as drug product (Lot ----------) stored at --°C (Lot -----) for --- months. ---------- was evaluated using the lot release ---------- assay (Method TM1065/B06547). Full-scale drug substance Lot ------ was tested after having been stored for --- months at ----°C and subjected to a total of three ---------- cycles. Drug product lot ---------- was tested after storage for ---months at --°C. Figure 1 shows the oligosaccharide profile of rhAPC drug substance Lot ------- at initial and after storage for 18 months at approximately ----°C. The ---------- (calculated as described in Method TM1065/B06547) and calculated ----------) are listed in Table 1. The ---------- are comparable between the initial and --- months samples and compare favorably with that of the rhAPC reference standard ----------. These results demonstrate that the rhAPC ---------- is stable

throughout the storage period. Based on known properties of N-linked glycoforms the most likely change in ---------- one might observe during storage would be ------ ----------. A decrease in ---------- content would be reflected in a ----------
----------, ----------), and a corresponding reduction in ---------- No such changes were observed, thereby demonstrating that ---------- is not lost from the rhAPC drug substance during storage nor during ----------

Figure 2 shows the ---------- for rhAPC drug product Lot ---------- after storage at --°C for ---- months. The ---------- are provided in Table 2. The data obtained for the drug substance lots used to produce drug product lot ---------- (lots ---------- and ----------) are also provided in Table 2 for comparison purposes. These data demonstrate that the ---------- and ---------- for rhAPC drug product stored for --- months at --°C are comparable to that of the rhAPC reference standard as well as typical rhAPC drug substance lots. Hence neither the drug product (fill finish) manufacturing process nor storage at --°C for --- months has a significant impact on the ---------- of rhAPC.
To provide further assurance that ---------- of rhAPC drug product remains consistent a ---------- test will be developed and implemented as a lot release assay by September 1, 2002.

To provide further assurance that ---------- of rhAPC drug product remains consistent a ---------- test will be developed and implemented as a lot release assay by September 1, 2002.

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Eli Lilly and Company also commits to adding ---------- on stability studies for both the drug substance and drug product. The specific tests for ---------- will be the ---------- for drug substance and the ---------- for drug product at both the --- and --- month timepoints. The drug substance lot using the revised stability protocol will be placed on stability study by February 1, 2002. The drug product lot using the revised stability protocol will be placed on stability after the ---------- method is validated for the drug product. The drug product ---------- assay will be submitted by September 1, 2002. The drug product lot will be placed on the annual stability program by February 1, 2003. The revised stability protocols for the drug substance and the drug product are provided below.

Stability Protocol for Representative Drug Substance Lots

The frequency of stability testing for routine lots of rhAPC drug substance is ----------

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----------. If a manufacturing change or deviation occurs and it is deemed necessary, additional stability testing will be undertaken. The protocol is as follows:

Stability Protocol for Representative Drug Product Lots

---------- drug product ---- of both the 5 and 20-mg presentations will be placed annually on stability according to the Stability Protocol for Representative Lots. If a manufacturing change or deviation occurs and it is deemed necessary, additional stability testing will be undertaken. The protocol is as follows:

The stability data will be reported in the annual report as required in CFR§314.81(b)(2)(iv).

Eli Lilly and Company will continue to monitor the drug product for potential changes in the degradation products. If a change or deviation occurs and it is deemed necessary, additional stability testing will be undertaken. Based on sound scientific principles and after proper review and approval, time points and/or tests may be added to the stability protocol.

Should any lot of rhAPC drug product fail to meet product specifications during the approved dating period, Eli Lilly and Company will withdraw the lot from the marketplace. A thorough investigation will follow any product withdrawal.

Reviewer's Conclusion:

This response is acceptable.

Question 8c

In the validation studies for the -------- potency test used for ------- ---------- and drug product, the information provided regarding specificity is minimal. Since the activity is measured by the ----------), more information regarding what potential contaminants could interfere with this assay is critical. Please provide additional information on the specificity of this assay and specifically address the question of whether any ---------- will interfere with the assay and whether any ---------- may interfere as well.

Lilly Response

Additional specificity studies have been initiated. The requested specificity information will be provided by January 1, 2002.

Reviewer's Conclusion:

This response is acceptable.

Question 8d

Only ------ data points are used to generate the standard curve for the ---------- assay and therefore, it is not possible to be absolutely confident that the linear part of the standard curve is being utilized in each analysis. Please utilize a standard curve in this assay which is generated from more than ------ data points.

Lilly Response

The ---------- test will be revised and validated to utilize a standard curve comprised of more than ----- data points. The new test method will be implemented by 1 September 2002.

This method will be submitted to the BLA as a "Supplement-Changes Being Effected" as required by 21 CFR601.12(c).

Reviewer's Conclusion:

This response is acceptable.

Question 8e

Please reevaluate drug substance and drug product release specifications when sufficient commercial lots have been manufactured. Please define the number of commercial lots that will trigger such a reevaluation. Please note that the acceptance criteria should be based upon manufacturing experience.

Lilly Response

Eli Lilly and Company has established the acceptance criteria for the drug substance and drug product upon both batch release and stability data. These criteria ensure that the specifications reflect the expected process variation and subsequent changes in the corresponding analytical property throughout the expiry period.

A re-evaluation of release specifications for drug substance and drug product will be performed when the stability studies from the corresponding process validations are completed through --- months, which is the final time point of the stability protocol as provided in Section I.H.1., Drug Substance Stability Protocol, for the drug substance and II.H.2., Future Stability Protocol, for the drug product. In addition, the release data for a minimum of --- commercial lots for the drug substance and at least --- lots from both strengths of the drug product will be included in this re-evaluation.

Eli Lilly and Company commits to submitting the re-evaluation of the drug substance release specifications to the FDA per 21 CFR 601.12(c), "Changes requiring supplement submission at least 30 days prior to distribution of the product made using the change" by May 1, 2002. The re-evaluation of release specifications for the drug product will be completed after --- months of stability data have been collected on the process validation lots and release data from at least --- lots of the 5 and 20-mg drug product presentation. This information will be submitted to the FDA per 21 CFR 601.12(c), "Changes requiring supplement submission at least 30 days prior to distribution of the product made using the change" by February 1, 2004.

Reviewer's Conclusion:

This response is acceptable.

Question 8f

Please implement routine testing of the ------ and ------ media, and the ---------- Solution ---------- , and other parameters as appropriate. Please provide specifications for this testing.

Lilly Response

The specifications for the ------ media are:

---------- Solution used in cell banking is made up at the time of use by combining ---------- medium (specifications provided above), ----------. Both ---------- and ---------- are controlled according to the specifications provided in the initial BLA, Section I.C.1.a.1., Specifications and Test for Purchased Raw Materials. Based on the specifications for the ------ media, the ---------- and ---------- provided in the BLA, Eli Lilly and Company believes that the ---------- Solution is adequately controlled based on its preparation as required.

Reviewer's Conclusion:

This response is acceptable.